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**PNPASE regulates RNA import into mitochondria.**

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**Public Summary:**

**Scientific Abstract:**

RNA import into mammalian mitochondria is considered essential for replication, transcription, and translation of the mitochondrial genome but the pathway(s) and factors that control this import are poorly understood. Previously, we localized polynucleotide phosphorylase (PNPASE), a 3' → 5' exoribonuclease and poly-A polymerase, in the mitochondrial intermembrane space, a location lacking resident RNAs. Here, we show a new role for PNPASE in regulating the import of nuclear-encoded RNAs into the mitochondrial matrix. PNPASE reduction impaired mitochondrial RNA processing and polycistronic transcripts accumulated. Augmented import of RNase P, 5S rRNA, and MRP RNAs depended on PNPASE expression and PNPASE-imported RNA interactions were identified. PNPASE RNA processing and import activities were separable and a mitochondrial RNA targeting signal was isolated that enabled RNA import in a PNPASE-dependent manner. Combined, these data strongly support an unanticipated role for PNPASE in mediating the translocation of RNAs into mitochondria.

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